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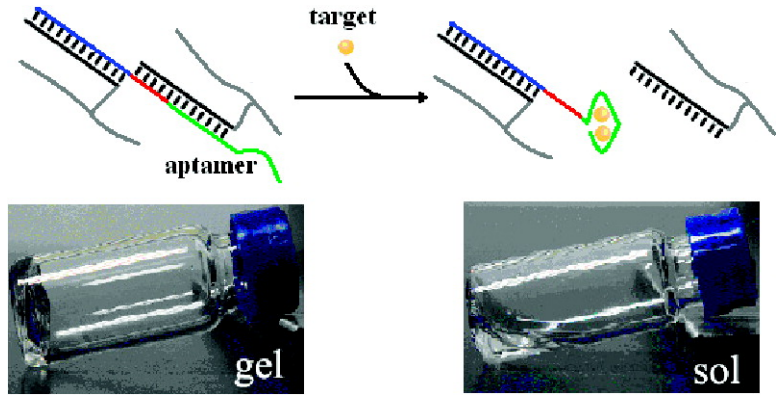

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Engineering Target-Responsive Hydrogels Based on Aptamer−Target Interactions

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We have molecularly engineered target-responsive hydrogels based on DNA aptamers for controllable release. A hydrogel is a network of polymer chains that are water-insoluble. Hydrogels are superabsorbent and possess a degree of flexibility very similar to natural tissue. Most important to this study, environmentally sensitive hydrogels have the ability to sense changes of pH, temperature, or the concentration of metabolite and release their load as a result of such a change. Thus, hydrogels that undergo physicochemical changes in response to applied stimuli, such as biomolecular binding, are promising materials for drug delivery and tissue engineering. Recent research has proven that hydrogels with such additional functionalities offer highly specific bioresponsiveness.[1−5] For example, hydrogels that undergo physicochemical changes via antibody−antigen[1] and DNA−DNA[2] interactions have been used as drug delivery devices and biosensors. However, in order to design bioresponsive hydrogels able to produce a given expected change, a comprehensive knowledge of the biological−material interactions is required. While current designs mainly focus on small molecules or peptides via receptor/ligand interactions, the lack of an appropriate screening method has often limited the effectiveness of these approaches.

Here we report an interesting approach for engineering such highly selective target-responsive hydrogels. The basic principle is that operating such bioresponsive hydrogels requires a change in their cross-linking density in response to targets. Therefore, our approach is based on the use of DNA aptamers that cross-link with linear polyacrylamide chains. Aptamers are single-stranded oligonucleotides that can specifically bind to their targets,[6] enabling them to selectively recognize a variety of molecules ranging from macromolecules to small compounds. In comparison with antibodies, aptamers, particularly DNA aptamers, are relatively easy to obtain and easily adaptable to modification.[7] Since the use of the DNA aptamer as cross-linker meets the requirements in terms of hydrogel cross-linking density, as noted above, hydrogels can be programmed to respond to a wide variety of targets.

We first constructed an adenosine-responsive polyacrylamide hydrogel. As shown in Figure 1, two acrydite-modified oligonucleotides, Strand A and Strand B, are separately copolymerized with acrylamide (4%, w/v) and thereby incorporated into the polyacrylamide chains. Detailed DNA sequences and linkages are shown in Figure 1b. The acrydite-modified oligonucleotides are reported to exhibit activity similar to that of acrylamide monomers, and oligonucleotide-based and switchable polyacrylamide hydrogels have recently been produced by this method.[2] By mixing these two oligonucleotide-incorporated polyacrylamide solutions in stoichiometric concentrations, we obtain a fluid system. This system can then be gelled by the addition of rationally designed cross-linking oligonucleotides, termed LinkerAdap.

LinkerAdap can be divided into three segments. The first segment (in blue) can hybridize with Strand A. The second segment (in red) can hybridize with the last five nucleotides of Strand B. The third segment (in green), which is the aptamer sequence for adenosine, can hybridize with the seven nucleotides on Strand B. In the presence of adenosine, the aptamer will bind adenosine. As a result, only five base pairs are left to hybridize with Strand B, which is unstable at room temperature. Therefore, Strand B will dissociate from the LinkerAdap, resulting in the dissolution of the hydrogel. The design of adenosine-induced dissolution was adapted from the recent reports of the Lu group, who used DNA base-pairing interactions and adenosine/aptamer interaction to assemble and disassemble gold nanoparticle aggregates for colorimetric adenosine sensors.[8]

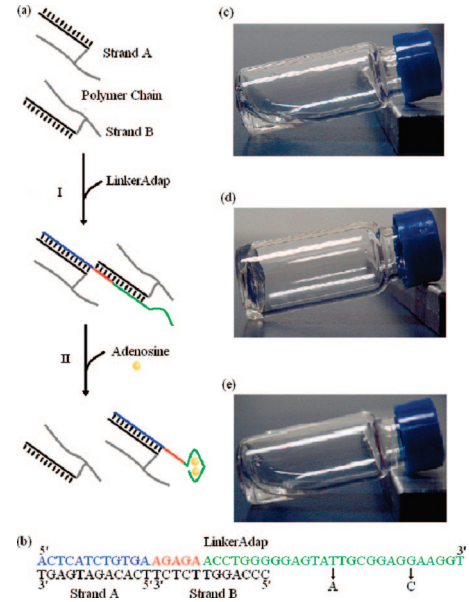

Figure 1. (a) Scheme of DNA-induced formation and adenosine-induced dissolution of hydrogel: (I) Add LinkerAdap to bind to Strand A- and Strand B-incorporated polyacrylamide mixture to form the hydrogel. (II) Add adenosine to competitively bind LinkerAdap to dissolve the hydrogel. (b) DNA sequences and linkages in the hydrogel. Optical images of the sol−gel transition: (c) system in the fluid state before LinkerAdap was added; (d) system in the gel state after LinkerAdap was added; and (e) system reverting to the initial fluid state after adenosine was added. In a control experiment, a mutated linker with the two mutations, as shown in (b) by the two short black arrows, was used.

The sol-to-gel and gel-to-sol transitions were examined through the flow behavior. Figure 1c shows that the system of chain A- and chain B-incorporated polyacrylamide mixture was in the fluid state. However, after the LinkerAdap was added, the system gelled (Figure 1d). By further addition of the adenosine with an excess amount relative to Strand A or Strand B, the hydrogel reverted to the initial fluid state (Figure 1e).

A control experiment was performed to support the mechanism of gel dissolution shown in Figure 1. Instead of the original linker

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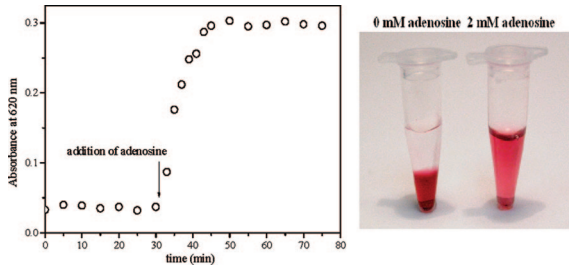
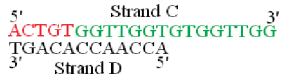


Figure 2. Absorption measurements of gold NPs in the gel systems. (Left) Cross-linked hydrogel with entrapped gold NPs disassembles upon addition of the target adenosine and dispenses the gold NPs into the buffer solution. There was little absorption in the beginning because the gold NPs are trapped inside the gel. Increasing absorbance was monitored after adenosine was added to the gel system as the gold NPs were released from the gel into the solution. The absorption was obtained with a spectrometer in constant-wavelength mode. (Right) Photograph of gels with entrapped gold NP is on the left, and the one on the right is the system 15 min after the addition of adenosine.

Scheme 1



(LinkerAdap), a mutated linker was used to form the hydrogel. The sites of mutation are marked in Figure 1b; aptamers with this mutated sequence were shown to be incapable of binding adenosine.[8] Hence, no gel-to-sol transition was observed for hydrogel linked by the mutated linker after adding 2 mM adenosine solution, which suggested that the observed gel-to-sol transition described above was indeed caused by adenosine/aptamer interactions.

The above results indicate that hydrogel cross-linked with aptamer DNA can be dissolved in the presence of adenosine. Consistent with the nature and properties of hydrogels as noted above, this further suggested that hydrogels could trigger controllable release of encapsulated molecules, including drugs. To demonstrate this principle, we used water-soluble citrate-modified 13 nm gold nanoparticles (NPs) as a model drug. Gold nanostructures can be easily tracked with IR absorption, and they were recently proved to have characteristics adaptable to photothermal therapy, making them ideal for testing biomedical applications.[9]

Accordingly, we prepared a cross-linked gel loaded with a high concentration of gold NPs. This gel was placed in a buffer solution for 12 h. As a consequence of the tight trapping of the NPs in the gel matrix, no increase in absorption due to release of NPs into solution could be detected. After the addition of 2 mM adenosine, however, an increase of absorbance in the solution surrounding the gel could be observed within several minutes, which indicated the release of the trapped gold nanoparticles and dissolution of the gel triggered by adenosine (Figure 2). It was further observed that the rate of NP release increases with increasing adenosine concentration (Supporting Information), whereas no NP release was detected for other ribonucleosides, such as cytidine, uridine, and guanosine. This suggests that the high selectivity of the aptamer was maintained in our gel system. The sensitivity of this controllable release is also investigated. Fifty micromolar adenosine can release detectable gold NPs from the hydrogel (Supporting Information).

To illustrate the generality of this method, we tested our strategy on a different type of target by constructing a hydrogel based on a reported human thrombin aptamer.[10] Detailed DNA sequences and linkages are shown in Scheme 1. To simplify the design, two acrydite-modified oligonucleotides, Strand C and Strand D, were used to construct the cross-linker. Strand C can be divided into two segments. The first segment (in red) can hybridize with the last five nucleotides of Strand D. The second segment (in green), which is the aptamer sequence for thrombin, can hybridize with the other seven nucleotides on the Strand D.[10] Mixing these two oligonucleotide-incorporated polyacrylamide solutions in stoichiometric concentrations yields a hydrogel directly. Similar to adenosine-responsive hydrogel, addition of thrombin to this hydrogel transforms the system into a fluid state. However, the release process of the thrombin-induced gold NPs is slower (130 min for 90% release) than the adenosine-induced release, which most likely results from the slow diffusion of thrombin in the gel (see Supporting Information).

In conclusion, we have demonstrated a general method for fast and easy engineering of target-responsive hydrogels based on aptamers. The hydrogels contain rationally designed DNA aptamer as the cross-linker. Because of this, competitive binding of target to the aptamer causes the decrease of cross-linking density and therefore dissolution of the hydrogel for potential drug release and other applications. Since aptamers have been obtained for a broad range of targets, including several cancer biomarkers,[11] and can be assembled,[12] this gel-to-sol transition system should have an equally broad spectrum of applications. Specifically, by conducting an experiment with water-soluble 13 nm gold nanoparticles as a model drug, we demonstrated that hydrogels could trigger controllable release of encapsulated molecules or nanomaterials and drugs. Thus, we believe that this method can be adapted for use in the selective release of therapeutic agents in specific environments where targets are found, thus creating a highly selective controllable release system. In addition, recent development of functional DNA (aptamers, DNAzymes, and aptazymes) should further extend the applications of this strategy.

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Supporting Information Available: Experimental procedures and the thrombin-induced gold nanoparticle release from hydrogel. This material is available free of charge via the Internet at http://pubs.acs.org.

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